AVAILABILITY OF CALCIUM AND PHOSPHORUS FROM FORAGES FOR HIGH PRODUCING AND DRY DAIRY COWS

TERMINATION REPORT

COOPERATIVE AGREEMENT

(USDA-SEA-AR-58-519B-9-839)

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INTRODUCTION

The availability of minerals in forages used in the diets of dairy cows is a major concern in dairy nutrition. Whereas considerable recent research has lead to new knowledge about dry matter digestibility, energy utilization and protein (nitrogen) metabolism, there has been less contemporary emphasis upon mineral metabolism in general and about utilization of the minerals in forages in particular.

Early research on calcium (Ca) and phosphorus (P) nutrition of dairy cattle involved work to determine requirements (Huffman, 1934; Ellenberger et al., 1932), about which there was considerable controversy. Requirements (NRC) were later reviewed by Reid (1962) and Hibbs (1963) and suggested to be too low. Current requirements are set at I.6 g Ca and .8 q P per 100 kg of body weight for maintenance; for milk production, 2.70 g Ca and 1.80 g P are recommended per kg of 4.0% fat milk (NRC, 1978). These recommended levels of Ca and P are, however, based upon certain assumptions of availability and endogenous secretion. These assumptions leave much to be desired when one attempts to balance rations, realizing that availability could vary extensively. For example, an average sized dairy cow producing 32 kg of milk requires about 100 g of Ca daily. Consumption of 11 kg of alfalfa containing 12 Ca g/kg (1.20%) of dry matter leads to a gross intake of about 132 g of calcium. The assumptions that one makes for the availability of calcium in the forage in this situation affects amount of supplementation needed. Thus, as availability is alleged to vary from 30 to 70%, there is 40 to 92 g of Ca available and 8 to 60 g of Ca needed for supplementation. Complicating this issue is the assumption that availability of Ca and P from forages, not unlike that of dry matter digestibility, is affected by many animal and plant factors and thus is not fixed.

Recent studies have raised questions about availability of Ca and P in feeds. Ammerman et al. (1957); Witt and Owen (1983); and Wise et al. (1961) demonstrated that availability of phosphorus varied widely among phosphorus supplements. Ward et al. (1972) determined the Ca and P balance of 45 lactating dairy cows over one or more lactations. They concluded from their data and data of others that the calcium

requirement for milk should be 5 g/kg of milk and that phosphorus should be 2.3 g/kg of milk, about twice current recommendations (NRC, 1978). Garces and Evans (1971) showed that calcium absorption in ruminants declined with age. Verdaris and Evans (1974a,b) showed that during early and mid lactation cows fed low levels of dietary calcium (.25% of dry matter) had lower (negative) calcium balance and lower milk production. Yoon and Evans (1981) reported that higher dietary concentrations of Ca resulted in greater true Ca absorption, increased milk yield and improved dietary efficiency. High producing cows were used and Yoon and Evans (1981) suggested that NRC recommendations should be increased 30 to 40%. Ward et al. (1979) raised questions about the availability of calcium in alfalfa, suggesting that much of it is in the form of insoluble calcium oxalate and therefore unavailable to ruminants. Availability, if indeed very low in certain forages, directly affects supplementation and dietary balancing. Adams (1975) reported that there is tremendous variation in the concentration of Ca and P, as well as most other minerals in feeds analyzed at the Penn State forage testing facility, suggesting that factors relating mineral content utilization and dietary balancing are significant and real. Duncan (1958) summarized data from many Ca and P balance trials in cattle and sheep. He concluded that there are many differences among trials, such as level of intake of Ca and P, ages of animals, species, feed Ca and P contents, etc; this makes it difficult to draw certain basic assumptions about availability, endogenous losses, digestibility, etc. Retention estimates from collections often lead to overestimates of body pools of Ca and P (Duncan, 1958). Among trials methods vary greatly, which can jeopardize accuracy and interpretation, particularly in low dietary mineral concentrations and short-term trials. Miller (1978) pointed out that in order to maintain homeostasis various approaches are followed by the animal, which include altering absorption, excretion, secretion and/or deposition. Animals may physiologically manipulate dietary mineral patterns to meet needs; the routes used to manipulate may depend upon various factors such as intake level, mineral content, etc., as well as the specific mineral. Miller (1975) further points out problems in mineral research

including statistical errors, means of isotope dosing, low mineral concentration in test diets, species of animals and genetic differences within species. Wasserman (1960) summarized that the interactions among calcium, phosphorus and other nutrients were complex and related to other dietary constituents as well as each other. Scott and McLean (1981) reviewed factors that control mineral absorption in ruminants. Calcium is controlled by absorption from the gut and thus via fecal excretion as well as urinary excretion. Phosphorus normally is low in the urine and is recycled via saliva. Gut absorption/resorption plays the major role in absorption/retention. The contribution of genetics to mineral metabolism could be very significant but has been greatly ignored. Wiener (1971) concluded that genetic factors are implicated in certain mineral disorders and body mineral concentrations of cattle. Only a few minerals have been studied in relation to genetic interactions and little is known about complete contribution of genetics to mineral utilization.

Minerals are important nutrients that have to be provided in adequate levels for optimal animal health and productivity. The adverse effects of high calcium intake upon animal performance and health are striking (Ricketts, et al., 1970; Beitz et al., 1974; Krook et al., 1971). The effects of low phosphorus upon animal health and growth are also well known (Forbes and Johnson, 1939). Costs to farmers are hard to determine but could conceivably be large. Unnecessary mineral supplementation of diets and/or feeding free choice minerals both are expensive practices and may also jeopardize animal health and productivity (Coppock et al., 1972; Muller et al., 1977). On the other hand, availability of forage minerals probably varies widely, due to many animal, plant and environmental factors. Because forages are the major feed source of dairy cattle, assumptions about average mineral availability can (and probably have) lead to many applied instances of over or under supplementation. For example, assuming that Ca of alfalfa is 50% available and supplementing appropriately could lead to a Ca imbalance if the availability were instead 30% or 70%. Such imbalances could explain unexpected, long term and latently poor performance of animals consuming diets that otherwise appear adequate.

There appears to be only a few programs in the United States actively pursuing research in mineral metabolism of dairy cattle. The Dairy Science Department of the University of Missouri has unique resources that were important in establishing and are important in maintaining a forage mineral research program as a satellite cluster of the United States Dairy Forage Program. These resources include new research laboratories and animal research facilities (Animal Sciences Center, completed Summer, 1983), intensive metabolic facility, Whole Body Counter, Research Reactor, Trace Substance Laboratory, Environmental Chambers and forage handling facilities.The Dairy Science Department has a history of dairy forage research, with investigations into fiber digestion, forage markers, whole body counting, nutrient x environmental stress, selenium uptake, mineral availability, nutrient digestibility and energy metabolism.

Because of these facilities, resources and the necessary personnel, a cooperative research agreement with USDA-SEA was approved and initiated in July, 1979. This report summarizes the research and findings of this cooperative agreement (58-519B-9-839).

OBJECTIVES

A major objective of this study was to develop a technique or techniques to measure availability of forage minerals, particularly Ca and P. Minerals were to be from natural sources - i.e., found within plant material, and in as natural a state as possible, as compared to inorganic (supplemental) forms. The mineral contributed by the forage part of the diet apparently is inseparable from the total pool of minerals derived from all dietary ingredients. Therefore, the minerals contributed by forage would appear to have to be marked for tracing when fed to animals. Once a method is established for marking forage Ca and P, it will be used along with conventional methods to study uptake, endogenous secretion, body pools, turnover and mineral interactions. Data will be collected to fit into a model. An initial base of information from balance trials in non-marked, cold diets fed to lactating cows will be established.

RESULTS AND DISCUSSION

Part 1. Preparing Forage Plants Intrinsically Labeled with Calcium and Phosphorus

a) Neutron Activation

Labeling forage plants by neutron activation was expected to be the most viable method of labeling plant Ca and P. This would result in a material similar in organic content and in biological nature to native forage. Digestion, passage, uptake of mineral and excretion of mineral should be similar to unmarked forage plants. Another advantage was that neutron activation could be performed at the research reactor facilities (on the UMC campus). The expediency of this was important, particularly in that the forage should be native and that radioactive decay would be less of a logistical problem, compared to purchase of label from a commercial supplier.

Forage plants were subjected to neutron activation at the UMC Research Reactor. Sufficiently high counts (dpm) were attained that both Ca and P apparently could be traced effectively. A major complication became readily apparent in that the organic matrix of the forage plants was oxidized during the activation process. This was considered to be highly undesirable because the organic matrix probably affects mineral utilization by the animal; lack of the matrix would likely result in data that do not reflect mineral utilization in native forage plants. Furthermore, the inorganic material remaining after neutron activation, although containing adequate levels of radioactivity, might be complexed in such a way that it would not reflect utilization of inorganic mineral supplements. Therefore, the neutron activation approach was not studied further.

b) Growing Labeled Forage Plants

A second approach was followed - growing native forage plants for intrinsic labeling of Ca and P. This ultimately was a lengthy process that involved (1) renovation and repair of a growth chamber; (2) establishing optimal light and temperature conditions for growth; (3) obtaining dormant plant roots, storing them and getting them to grow hydroponically; and (4) comparing hydroponic plants to native forage

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plants. Initially we attempted to grow forage plants in various greenhouses and at our animal metabolic facility. We could not adequately control temperature, light, relative humidity and other environmental factors and subsequently grown plants were dissimilar enough in botanical and nutrient composition to warrant better growing facilities and conditions. Thus, we attempted to obtain use of a growth chamber for growing our forage plants under more controlled and optimal conditions.

Obtaining the use of a growth term chamber for longterm plant growth and lableing was not feasible, because of limited space in present chambers; it was not possible to get secure space for longterm use. Use of isotopes and the control necessary to contain radioactive materials complicated the situation. It became apparent that renovation of an existing enviornmental chamber, previously used for small ruminant metabolism, was our best alternative. This was modified and adapted for growth of our forage plants. Some of the renovation involved temperature and relative humidity equipment; a significant effort centered around an effective and controlled lighting system. Initially we were uncertain about temperature and light/dark conditions for best plant growth. From initial growth work in the greenhouse trials and from advice of colleagues in Agronomy, we tried 14 hours daylight/10 hours of darkness. Temperatures were held at 30C and 25C, respectively, during these periods. Plants grown under these conditions were not satisfactory; they were spindley, weak-stemmed and hard to grow. It was obvious that these conditions had to be changed; we decreased temperature to 25C throughout the day and night. Plants produced under these conditions appeared normal in appearance and morphology; these conditions were therefore maintained throughout the rest of our studies. Our light source (fluorescent) was suspended 36" above the plant pot tops. Light intensity at the pot top level was about 100-160 watts/in^2/sec: 12" from the light, intensity was about 300-500 watts/m^2/sec. For reference, intensity is about 500 watts/m^2/sec outside on a cloudy day.

Another problem that had to be solved was getting plant material to grow in the chamber at selected times so that the same vegetative stage would be available

for animal feeding trials as they occurred. We did not want different animal trials or different phases of a given trial to have different vegetative stages and therefore qualities as a source of variation. In the early greenhouse trials we had obtained fescue and alfalfa roots from various forage fields, stored them at -4C, replanted them and obtained new vegetative growth. We thus could produce for labeling a vegetative material that would be at the same harvest stage and quality as needed for various animal feeding trials.

We grew our plants hydroponically, versus growth in sand or soil, so that elements such as silica and aluminum would not complicate forage quality (i.e., mineral complexing and in vivo digestibility). The hydroponic solution used (table 1) was Hoagland's Solution #2, as modified by Johnson et al. (1957). Water (distilled) was added to each hydroponic pot once daily during early stages of plant growth; this was increased to 3 times daily as plants became larger and more mature. Nutrient solution was added as needed (as determined by early nutrient analysis of vegetative growth), which was every 4-10 days depending upon plant size and maturity. Buffer was also added to the hydroponic solution to control pH; this was done daily, as needed. Our general approach was to supplement the existing hydroponic nutrient solution rather than discard and renew daily. The rationale for this was that use of labeled mineral for uptake would preclude discarding of nutrient solutions. It seemed logical that we should strive to grow plants in the same (supplemental) type of regime.

Using this chamber and the growing conditions thus described we can grow and have grown plants successfully; we have been able to produce forage containing labeled Ca and P. We purchase labeled Ca and P from a commercial supplier at a prescribed time and isotopic activity level so that plants will have adequate activity, allowing for decay. We add the radioactive Ca and P to the nutrient solution starting with early vegetative growth and obtain uptakes of activity of about 50%. Plant growth is synchronized with animal feeding trials such that the labeled forage is available for a given trial is as similar as possible that used in other trials. It is important that both forage growth stage (quality) and radioactivity be closely controlled and

synchronized with each feeding trial to attempt to control variation due to forage characteristics.

Part 2. Comparison of Hydroponically Grown Forage to Native Forage

We were very concerned that hydroponically grown forage plants might not be anatomically similar to native forage and that fiber, dry matter digestibility, mineral content and mineral availability also would not be similar to that of native forage. Consequently, we characterized hydroponically grown forage plants during the plant growth experiments. Plants were analyzed for anatomical components, and fiber, calcium and phosphorus concentrations. Plants analyzed included those grown in the early (greenhouse) trials as well as later trials in the renovated growth chamber. Means reflect data from about 10 trials and, therefore, represent a range of time, growing conditions, human experiences, etc. The data are presented in table 2. Leaf and stem proportions were not greatly different between native and hydroponic forage. Detergent fiber was generally lower in the hydroponic forage, especially fescue. This did not appear to be a major problem, but it was more deviant than originally expected. Hydroponic and native alfalfa plants were more similar to each other in fiber concentration than fescues. Mineral content (Ca and P) was consistently higher in hydroponic fescue than native forage. Hydroponic alfalfa had lower Ca content than native, whereas P content of hydroponic alfalfa was higher than native alfalfa. We were able to alter the anatomical and nutrient composition of the hydroponic forages as we gained experience. Thus, plant yield increased from 1.50 and 1.33 g of fescue and alfalfa DM per pot, respectively, to 3.11 and 5.10 g/pot. Also, leaf/stem ratios, detergent fiber, Ca and P were likewise altered (tables 2 and 3). Based upon data summarized in tables 2 and 3 and other data in progress, it appeared that currently grown hydroponic forage is similar enough to native to serve our needs. There were some variations in mineral content compared to normal forage, probably because hydroponically grown forage takes up mineral in patterns different from native forage. Fiber varied also but did not appear to potentially cause problems. More importantly, we are able to repeatedly produce forage at about the same vegetative stage and in

synchrony with needs for feeding trials.

Inorganic residues remaining after ashing ADF (ADF-ash) were a concern; silica was expected to be a major constituent in native grown forage. Fescue tends to range from 1-3% in silica, whereas native grown alfalfa usually contains less than .5% silica (in the ADF-ash). Because the hydroponic nutrient solution contained no silica, hydroponically grown forages contained very low levels of silica (as ADF-ash). Fescue contained between 0 and .5%, while alfalfa contained between 0 and .3%. Thus, silica did not appear to confound our test forages. Another inorganic interaction of suspicion was oxalate, which can insolubilize various cations, including Ca. Analyses are continuing; we have had a technical problem with analyses of forage plants for oxalate content and some data may have been erroneous. At this point, oxalate content appears to range from .8 to 1.2% of the forage (species not affected so far).

We have analyzed some hydroponics for various macro and micro elements (table 4). Although there is considerable variation among and within species, values generally appear in the range normally expected. Potassium content has been higher than normally observed for most native forage, while the other elements appear to be similar.

In another set of experiments we digested the ADF fraction of native and hydroponically grown forages using an _in vitro_ procedure (Goering and Van Soest, 1970). The major objective was to ascertain that lignocellulose (ADF), the major contributor to forage dry matter digestibility (or indigestibility), was similar in digestibility for hydroponically grown forage and native (table 5). There was a considerable amount of variation among determinations, which is normal in such a procedure; lignocellulose of native fescue was not digested as well as that of hydroponic (19.4 vs 39.8%). However, initial ADF of native forage was higher than hydroponic and this probably accounts for the lower digestibility. Hydroponic alfalfa leaves and stems also were more digestible (65.6 and 35.8%) those of than native forage (46.5 and 22.7%). In this case also lower initial ADF content (table 4) probably accounts for this difference. It is possible that lignocellulose of hydroponic forages (both fescue and alfalfa) may have been more digestible because silica content was low. High silica content is

thought to reduce digestibility because of attachment to cell wall (fiber). Silica content of forage can range from <1% to 23%, depending upon forage species and soil type (Van Soest, 1970). Silica content of our native forage ranged from less than 1% to about 7% of the DM. Much of the silica in native forage was associated with the leaves; with stems contained much less. Hydroponic forage, by comparison, contained less than 1% silica, with most plants containing less than .25%; highest concentrations again, were in the leaves.

Part 3. Animal Feeding Trials

a) Feeding of Labeled Forage

Two preliminary trials were completed with a non-lactating and a lactating cow to monitor excretion patterns of labeled mineral fed as part of labeled forage. In addition, the non-lactating cow was injected intravenously with labeled Ca and P. Excretion patterns and routes were similar to those reported by others. Our main concern was qualitative rather than quantitative aspects; we wanted to be certain that initial activities were sufficiently high to ensure detectable label in all possible excretion paths and to ensure that endogenous turnover could be monitored. We are about ready to commence a full scale, intensive feeding trial using 8 lactating cows. Labeled forages (alfalfa and fescue) will be used to supply labeled Ca and P. Excretion patterns, turnover, endogenous mineral loss, etc., will be determined during the dry period and during the early, mid and late portions of the subsequent lactation. This study should be completed in about one year and data will be reported as part of the objectives of our second Cooperative Agreement (USDA-SEA-AR 58-519B-1-992).

b) Balance Trial Using Lactating Cows

A balance trial was conducted using Holstein cows in mid lactation; effects of two dietary treatments (high soluble and low soluble protein) and two environmental treatments (thermoneutral and heat stress) upon mineral metabolism were determined. The two diets contained ground corn, corn silage, chopped alfalfa hay and either soybean meal, as the low soluble protein source (LS), or linseed meal, as the high soluble protein source (HS). The environmental treatments were thermoneutral (21C and 50%

relative humidity) and heat stress (31C and 50% relative humidity). The design was a replicated split plot; the two diets were used in two replicates of 6 cows each (3 per diet). Environmental treatments were sequential - two weeks at thermoneutral; two weeks at heat stress and two weeks at thermoneutral. Cows in each dietary treatment were subjected to all three environmental treatments. Prior to the beginning of the trial the cows were brought from the farm to the environmental chambers to 5 days of acclimatization. Feeds were offered ad libitum in two daily allotments; refusals were weighed daily. Cows were milked at 12 hour intervals using a portable bucket type milker; milk weights were recorded daily. Body weights were taken weekly. During the first 5 days the second week of each period, feces and urine were collected and weighed. Samples of feeds, feces, urine and milk were taken and composited for input/output measures and for determining digestibilities and balance.

The results of this trial are in tables 6 and 7. The diets were quite similar in most nutrients, except for soluble protein, which was by design higher in the HS diet. The HS diet was slightly lower in Ca and P content and slightly higher in fiber content than the LS diets. Concentration of each mineral in feces, urine and milk was quite similar between dietary treatments, except for a low urinary P content on the HS diets. The reason for this is not apparent. Dry feed intake was only slightly less for the HS diets (table 7). Consequently, many other parameters were similarly lower: i.e., fecal output, milk output, calcium in feed and feces, phosphorus balance. Several means were significantly different ($P<.05$), including Ca and P digestibility, Ca balance and P content of milk. Lowered digestibility of Ca and P on the HS diets was unexpected and intriguing. Since Ca intake and gut uptake of Ca are inversely related, the lower intake of Ca in the HS diets should be associated with higher Ca digestibility than the LS diets. However, such was not the case, suggesting a diet x mineral interaction, possibly with protein quality.

During heat stress feed intake was depressed, as would be expected. Consequently, other nutrients were subsequently affected, including fecal DM output, fecal Ca and P output and milk Ca and P. output. In the second thermoneutral period most measures were similar to those noted in the first thermoneutral period. During heat stress fecal

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Ca output and urinary P output decreased to a greater extent than can be explained by decreased mineral intake. The reason for this is not clear. During heat stress decreased Ca and P intake lead to a larger proportion being digested (35% of Ca and 42.0% of P), compared to 26% (Ca) and 36% (P) during thermoneutral. The absolute amounts digested were less during heat stress (53 vs 36 g/day for Ca and 29 vs 23 g/day for P). Calcium and phosphorus excreted in the urine (<3 g/day) was small, similar to other studies; most variations in Ca and P metabolism occurred at the gut level. Calcium balance decreased in heat stress and continued to decrease in the second thermoneutral period, whereas phosphorus balance went up slightly during heat stress and decreased slightly during the second thermoneutral.

SUMMARY

A method of hydroponically growing forage plants for intrinsically labeling Ca and P was developed, because neutron activation of native forage was not suitable. A growth chamber was renovated and equipped for controlling light, humidity and temperature, after greenhouse and other growing facilities were determined unsuitable or unavailable for forage growth. Forages (fescue and alfalfa) were grown hydroponically with 14 hours of light and 10 hours of dark; temperature was held at 25C during most growth studies. The resultant hydroponic forage, grown when needed and in phase with animal feeding trials, appeared to be generally similar to native forage, based on extensive botanical and chemical analyses. Uptake of labeled Ca and P, provided via the nutrient solution, was about 50% for both minerals and appeared to provide adequate activity for tracing mineral metabolism in the cow's body. Preliminary feeding trials suggest that excretion patterns were similar to those of other studies and were applicable to the trials planned.

A balance trial was conducted with 12 lactating cows fed two dietary protein qualities at thermoneutral, heat stress and thermoneutral conditions. Apparent digestibility of Ca and P were low (35 and 45%); balances of Ca and P were variable but positive. There were some apparent interactions of minerals with environmental stress and with diets.

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Table 1. Composition of Hoagland's Nutrient Solution (Modified by Johnson[a] et al.)

Salts used	Concentration of elements (μM)	
KNO_3	N	16000
$Ca(NO_3)_2 \cdot H_2O$	K	6000
$NH_4 H_2PO_4$	Ca	4000
$MgSO_4.7H_2O$	P	2000
KCL	S	1000
H_3BO_3	Mg	1000
$MnSO_4.7H_2O$	Cl	50
$CuSO_4.5H_2O$	B	25
H_2MoO_4	Mn	2.0
Fe-EDTA	Zn	2.0
	Cμ	0.5
	Mo	0.5
	Fe	20.0

[a]Johnson et al. (1957).

[9]Johnson et al. (1957).

Table 2. Comparison of Native and Hydroponically Grown Forages

Forage/portion	Anatomical separations		Acid detergent fiber		Calcium		Phosphorus	
	C[a]	H[b]	C	H	C	H	C	H
	% of dry weight							
Fescue								
Total	----	----	33.9	26.3	.863	1.07	.498	.644
Shoots	24.7	23.8	33.3	27.3	.449	.521	.476	.664
Stems	----	----	----	----	.132	----	----	----
Leaf tips	40.3	44.3	34.9	23.5	.900	1.68	.527	.558
Leaf base	34.9	31.9	32.9	26.6	.619	.921	.483	.567
Aftermath	----	----	49.0	20.9	----	----	----	----
Roots	----	----	----	----	----	.520	----	.826
Alfalfa								
Total	----	----	25.6	27.4	2.14	1.58	.269	.517
Leaf	52.9	55.3	16.7	16.3	3.53	2.04	.292	.428
Stem	47.1	44.7	46.1	38.1	----	.822	----	.462
Top	11.8	21.6	37.3	34.6	1.43	1.12	.257	.421
Mid	22.4	----	47.7	----	1.07	----	.208	----
Butt	13.0	19.8	52.3	51.4	.89	.916	.198	.312
Roots	----	----	----	----	----	1.02	----	1.15

[a]C = control, native forage.

[b]H = hydroponically grown forage.

Table 3. Comparison of Native and Hydroponically Grown Forages (Summary across 10 Trials)

	Hydroponic fescue		Control fescue	Hydroponic alfalfa		Control alfalfa
	Initial	Current		Initial	Current	
Yield DM, g/pot	1.50	3.11	----	1.33	5.10	----
Leaf/stems	----	59/41	----	----	43/57	----
ADF[b], % DM	28.4	32.0	33.9	27.2	37.4	25.6
Ca, % DM	.96	.71	.87	1.74	1.35	2.14
P, % DM	.76	.38	.50	.78	.41	.27

[a]DM = dry matter.

[b]ADF = acid detergent fiber.

Table 4. Macro and Micro Mineral Content of Hydroponically Grown Forages

		Alfalfa		
	Fescue	Leaf	Stem	Plant
K, % DM[a]	3.24	3.78	2.54	2.85
Mg, % DM	.24	.247	.102	----
Cu, ppm DM	11.6	15.2	4.3	9.8
Mn, ppm DM	22.0	25.3	8.6	17.0
Fe, ppm DM	101.2	119	57.7	88.0
Zn, ppm DM	23.6	39.2	24.3	32.0
Na, ppm DM	100.0	152	45.6	98.6

[a]DM = dry matter.

Table 5. In Vitro 48 hour Digestibility of Acid Detergent Fiber of Hydroponically Grown and Native Forages

	Initial ADF[a,b]	In vitro[c] digestibility of ADF (48 hr)
	% DM	%
Fescue		
Native	43.0	29.4
Hydroponic	32.6	39.8
Alfalfa		
Native (leaves)	22.3	46.5
(stems)	52.3	28.7
Hydroponic (leaves)	17.0	65.6
(stems)	49.0	35.8

[a]ADF = acid detergent fiber.

[b]Initial ADF = ADF content prior to in vitro digestion.

[c]Goering and Van Soest, 1970.

Native	13.0	23.4
Hydroponic	32.6	20.8
alfa		
Native (leaves)	22.3	16.5
(stems)	52.3	28.7
Hydroponic (leaves)	17.0	63.6
(stem)	19.0	33.8

acid detergent fiber.

ro ADI = ADF content prior to in vitro digestion.

Table 6. Diet Composition and Calcium and Phosphorus Content of Feeds, Feces, Urine and Milk of Protein Solubility Trial

	Diet	
	LS[a]	HS[b]
Feeds		
Acid detergent fiber, % DM[c]	19.1	21.3
Total protein, % DM	15.7	15.4
Soluble protein, % total	26.6	42.9
Net energy, Mcal/kg	1.50	1.51
Calcium, % DM	.907	.862
Phosphorus, % DM	.421	.410
Feces		
Calcium, % DM	1.92	1.88
Phosphorus, % DM	.757	.803
Urine		
Calcium, %	.0126	.0156
Phosphorus, %	.00921	.00384
Milk		
Calcium, %	.129	.122
Phosphorus, %	.0829	.0809

[a]LS = low soluble protein diet.

[b]HS = high soluble protein diet.

[c]DM = dry matter.

Table 7. Effects of Dietary Protein Solubility and Environmental Conditions upon Mineral Balance in Lactating Cows

	Diets		Period		
	LS	HS	TN1	HS	TN2
Dry feed intake, kg/d	16.8	15.3	19.2[a]	12.1[b]	17.1[c]
Fecal output, kg/d	5.57	5.30	6.66[a]	3.55[b]	6.09[c]
Urine output, kg/d	20.6	19.0	11.4[a]	32.3[b]	15.6[a]
Milk output, kg/d	24.1	21.3	26.5[a]	18.8[b]	22.9[c]
Ca intake, g/d	153.0	133.7	170.9[a]	105.0[b]	154.2[c]
Ca feces, g/d	104.3	99.8	118.2[a]	69.0[b]	118.9[a]
Ca urine, g/d	1.81	1.87	2.26[a]	1.48[b]	1.79[a,b]
Ca milk, g/d	30.9	26.0	33.1[a]	24.5[b]	27.9[c]
Ca digestibility, %	32.6[a]	25.7[b]	30.4[a]	34.8[a]	22.2[b]
Ca balance, g/d	16.0[a]	6.0[b]	17.4[a]	10.0[a,b]	5.58[b]
P intake, g/d	71.3	62.7	77.7[a]	52.7[b]	70.6[c]
P feces, g/d	41.4	41.2	48.6[a]	30.3[b]	45.0[a,b]
P urine, g/d	1.36	.556	.961[a,b]	.465[b]	1.46[a]
P milk, g/d	20.0[a]	17.0[b]	21.3[a]	14.8[b]	19.5[c]
P digestibility, %	41.8[a]	34.5[b]	36.1	42.6	35.7
P balance, g/d	8.50	3.94	6.89	7.10	4.67

[a,b]Means differ at P<.05 using LSD.

[a,b,c]Means differ at P<.05 using LSD.